Multiresidue Method for the Determination of Polychlorinated Dibenzo-*p*-dioxins, Polychlorinated Dibenzofurans and Non-ortho Substituted Polychlorinated Biphenyls in Wildlife Tissue by HRGC/HRMS

M. Simon and B.J. Wakeford

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PREFACE

The Canadian Wildlife Service's National Wildlife Research Centre (NWRC) has been providing analytical determinations of polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and non-ortho substituted polychlorinated biphenyls (NOPCBs) in wildlife tissues since the early 1980s, in support to its National Wildlife Toxicology Program. Protocols used prior to 1993 for the sample preparation, extraction and analysis of these contaminants using high resolution gas chromatography/ **low resolution mass spectrometry** (HRGC/LRMS) are described in publications listed in the References section of this report.

The method of analysis presented here has been used since 1993 for the determination of PCDDs/PCDFs and NOPCBs in various type of specimens such as avian egg, liver, breast muscle and plasma, using high resolution gas chromatography/**high resolution mass spectrometry** (HRGC/HRMS). The method is based on established techniques in the field in conjunction with the automated cleanup method developed in-house by Norstrom and Simon [see ref. 2.3] and high-resolution mass spectrometry. This report describes in detail the sample extraction and cleanup, and the separation, identification and quantitation of the analytes of interest.

Standard Operating Procedures (SOPs) specific to our organization are cited throughout this document. These SOPs are not readily available in the published literature but can be obtained from the authors, upon request.

The names of manufacturers, suppliers and trade names are included only to document the exact assay conditions adopted by the NWRC. Other equivalent products, instruments or reagents from other sources may also give satisfactory results.

PRÉFACE

Méthode multi-résidus pour le dosage des polychlorodibenzo-para-dioxines (PCDD), des polychlorodibenzofuranes (PCDF) et des biphényles polychlorés non-ortho substitués (NOPCB) dans des tissus d'espèces sauvages par chromatographie gazeuse à haute résolution (CGHR), couplée à la spectrométrie de masse à haute résolution (SMHR).

Depuis le début des années 1980, le Centre national de la recherche faunique (CNRF) du Service canadien de la faune fournit des analyses de polychlorodibenzo-*p*-dioxines (PCDD), de polychlorodibenzofuranes (PCDF) et de biphényles polychlorés non-ortho substitués (NOPCB), dans le cadre de son Programme national de surveillance des effets des produits toxiques sur les espèces sauvages. Les protocoles utilisés avant 1993 pour la préparation des échantillons, l'extraction et le dosage de ces contaminants, utilisant la chromatographie gazeuse à haute résolution couplée à **la spectrométrie de masse à faible résolution** (CGHR/SMFR) sont décrites dans les publications présentées à la section des références du présent document.

La méthode d'analyse qui suit a été utilisée depuis 1993 pour l'analyse des composés susmentionnés dans divers types d'échantillons tels que les oeufs, le foie, les muscles pectoraux et le plasma d'oiseaux, par chromatographie gazeuse à haute résolution **/spectrométrie de masse à haute résolution**. Elle est basée sur des techniques d'analyse conventionnelles dans le domaine, et incorpore la technique d'épuration des échantillons automatisée développée à l'interne par Norstrom et Simon [voir réf. 2.3] avec l'analyse par spectromètre à haute résolution. Ce rapport décrit en détail les étapes d'extraction et d'épuration des échantillons, et de séparation, d'identification et de quantification des composés qui nous intéressent.

Tout au long du document on fait référence à des modes opératoires normalisés («*SOPs*») qui sont spécifiques à notre organisation. Ces procédures ne sont pas disponibles dans la littérature mais peuvent être obtenues en communiquant directement avec les auteurs.

Le nom des manufacturiers, fournisseurs et nom de commerce des produits sont inclus uniquement dans le but de documenter les conditions d'analyse précises utilisées par le CNRF. Des produits, instruments ou réactifs équivalents provenant d'autres sources peuvent aussi donner des résultats satisfaisants.

ABBREVIATIONS

DCM	dichloromethane (methylene chloride)
GPC	gel-permeation chromatography
HRGC/HRMS	high resolution gas chromatography/high resolution mass spectrometry
HRGC/LRMS	high resolution gas chromatography/low resolution mass spectrometry
HxCDD/F	hexachloro dibenzo-*p*-dioxin/furan
HpCDD/F	heptachloro dibenzo-*p*-dioxin/furan
NOPCBs	non-ortho substituted polychlorinated biphenyls
OC	organochlorine
OCDD/F	octachlorodibenzodioxin/furan
PCDD	polychlorinated dibenzo-*p*-dioxin
PCDF	polychlorinated dibenzofuran
PFK	perfluorokerosene
SIM	selected ion monitoring
TCDD	tetrachloro dibenzo-*p*-dioxin

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TABLE OF CONTENTS

PREFACE ..... *i*

PRÉFACE ..... *ii*

ABBREVIATIONS ..... *iii*

ACKNOWLEDGEMENTS ..... *iv*

LIST OF TABLES ..... *vi*

LIST OF FIGURES ..... *vi*

1. SCOPE AND FIELD OF APPLICATION 1

2. REFERENCES 1

3. PRINCIPLES AND DEFINITIONS 2

4. REAGENTS, SOLUTIONS, MATERIALS AND STANDARDS 3

4.1 Reagents
4.2 Adsorbents for Sample Cleanup
4.3 Solutions
4.4 Stock Standards
4.5 Working Standards
4.6 QA Reference Material
4.7 Method Blank

5. AUXILIARY EQUIPMENT 10

5.1 Glassware and Labware
5.2 Equipment
5.3 Instrumentation

6. SPECIMEN OR SAMPLE HANDLING REQUIREMENTS 13

7. PROCEDURE 13

7.1 Columns Preparation
7.2 Extraction - Tissue samples
7.3 Extraction - Plasma
7.4 Gel Permeation Chromatography (GPC)
7.5 Alumina Column Cleanup
7.6 Carbon/Glass Fibre Column Separation
7.7 Florisil Column Separation
7.8 HRGC Operating Conditions

7.9 HRMS Operating Conditions
7.10 HRMS Calibration
7.11 Instrument Daily Calibration Verification
7.12 HRGC/HRMS Samples Analysis
7.13 Five-point Calibration Curve
7.14 Moisture Determination
7.15 Lipid Determination

8. EXPRESSION OF RESULTS 28

8.1 Calculation of Relative Response Factor (RRF)
8.2 Calculation of Analyte Concentration
8.3 Calculation of Recovery for $^{13}C_{12}$ Surrogate Standards

9. REPRESENTATIVE DOCUMENTS 30

10. QUALITY CONTROL 38

11. CRITICAL CONTROL POINTS 41

LIST OF TABLES

TABLE 1 - COMPOSITION OF PCDDs/PCDFs STANDARD SOLUTIONS FOR HRMS 7

TABLE 2 - COMPOSITION OF PCDDs/PCDFs CALIBRATION STANDARDS (CS) FOR HRMS 8

TABLE 3 - ELUTION ORDER OF PCDDs/PCDFs WINDOW DEFINING MIXTURE ON A 30 M DB5 COLUMN. 9

TABLE 4 - COMPOSITION OF NOPCB STANDARD SOLUTIONS FOR HRMS 9

TABLE 5 - INTERNAL CALIBRATION RESULT 31

TABLE 6 - DIOXIN FURAN ONE POINT CALIBRATION CURVE 32

TABLE 7 - RESULT TABLE 34

TABLE 8 - DIOXIN FURAN TARGET 35

TABLE 9 - DIOXIN FURAN FIVE-POINT CALIBRATION CURVE 36

LIST OF FIGURES

FIGURE 1 - GPC/CARBON COLUMN CHROMATOGRAPHY APPARATUS 12

FIGURE 2 - FLOW DIAGRAM OF EXTRACTION, CLEAN UP AND ANALYSIS OF PCDDs/PCDFs AND NOPCBs 27

FIGURE 3 - TUNING DATA FOR PCDDs/PCDFs 30

MULTIRESIDUE METHOD FOR THE DETERMINATION OF POLYCHLORINATED DIBENZO-*P*-DIOXINS, POLYCHLORINATED DIBENZOFURANS AND NON-ORTHO SUBSTITUTED POLYCHLORINATED BIPHENYLS IN WILDLIFE TISSUE BY HRGC/HRMS

1. SCOPE AND FIELD OF APPLICATION

This method is applicable to the analysis of PCDDs/PCDFs and NOPCBs in animal tissues. It has been used in our laboratory to determine the levels of these environmental contaminants in various tissue types (liver, muscle, whole body homogenates, eggs, plasma, etc.) from various species (birds, fish, mammals, etc.), with typical limits of detection of 0.1 to 0.2 ng/kg (wet weight basis). The method has not been validated for plant tissues or soils.

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3. PRINCIPLES AND DEFINITIONS

A representative portion of the sample is extracted with DCM/hexane and lipids and long-chain hydrocarbons are separated from OC compounds by GPC [2.1]. Further clean-up of the OC fraction is achieved by alumina column. PCDDS/PCDFs and NOPCBs are then separated from most OC compounds by adsorption on a carbon/glass-fibre column. After desorption with toluene, NOPCBs are separated from PCDDs/PCDFs by chromatography on Florisil column [2.4]. The analytes are identified and quantified by HRGC/HRMS using internal and external standards. The determination of lipid content is done by extracting a second portion of the sample with hexane and a third portion is used for the determination of moisture.

4. Reagents, Solutions, Materials and Standards

SAFETY PRECAUTIONS

⇒ The toxicity or carcinogenicity of each reagent and standard used in this method has not been precisely defined. Each chemical must be treated as a potential health hazard.

⇒ Operations with toluene, DCM, hexane and acetone should be performed in a fume hood and dermal contact with solvents should be avoided. It is permissible to wear polyethylene protective gloves, but surgical rubber gloves must not be used because the sample may become contaminated with phthalate esters.

⇒ Bottles of standard solutions which are used in sample preparation should not contain more than 1 µg of TCDD toxic equivalents [2.7]. These standards should always be opened and used in a fume hood and should be stored in a locked cabinet when not in use. PCDDs/PCDFs and NOPCBs standards are usually obtained already diluted to safe concentration. However, it is a good practice to check the concentration of these solutions against the previous batch of unlabeled standard solution, prior to routine use. Handling of these compounds must be done only by qualified technical staff in the medium hazard laboratory.

⇒ General safety rules and waste disposal procedures that apply to the Trace Organic Chemistry Laboratory must be followed (ref. Laboratory Safety Manual).

⇒ Material Safety Data Sheets (MSDSs) for the products used in the assay must be read.

4.1. Reagents

4.1.1. Acetone, Omnisolv®, BDH AX0142-1

4.1.2. Hexane, Omnisolv®, BDH HX0296-1

4.1.3. Dichloromethane, Omnisolv®, BDH DX0831-1

4.1.4. Methanol, Omnisolv®, BDH MX0488-1

4.1.5. Toluene, BDH TX0737-1

4.1.6. Formic acid, AnalaR®, BDH B10115

4.1.7. Sodium sulphate, anhydrous granular, (Na_2SO_4), BDH ACS85046

Wash 600 g of Na_2SO_4 in a glass column 3 cm ID x 50 cm long with 600 mL DCM/hexane (1:1), air dry in an open dish under the fume hood, heat 3 hours at 400°C, cool and transfer in a tightly capped glass bottle. ***Note:*** If, after heating, the sodium sulfate develops a greyish cast (due to the presence of carbon in the crystal matrix), discard that batch.

4.1.8. Envirobeads™ S-X3, Select (200-400 mesh), ATS Scientific 091-203 for GPC

4.1.9. Carbon, activated, super A, AX-21, Anderson Development Co., Adrian, MI, USA

4.1.10. De-ionized water from the Milli-RO / Milli-Q system (Millipore)

4.1.11. Helium, compressed bottled gas, Central Oxygen Ltd., HE UHP SG 103168K

4.1.12. Nitrogen, compressed bottled gas, Central Oxygen Ltd., N_2 PRE PURE SG 105411K

4.1.13. Air, compressed bottled gas, Central Oxygen Ltd., AIR EX-DRY SG 1001D7K

4.2. Adsorbents for Sample Cleanup

4.2.1. Alumina, basic, Brockman activity 1, 60-325 mesh, Fisher Scientific A941-500

Activate by heating at 100°C for 2 hours. Cool and then store in capped glass bottle. Place open bottle every week-end in oven at 100°C. ***Note:*** Prior to routine use, every new batch of alumina is tested for the elution of $^{13}C_{12}$-labeled PCDDs/PCDFs and NOPCBs, using method described in Section 7.5.

4.2.2. Florisil®, pesticide grade, 60-100 mesh, BDH B28722-38

Heat to 400°C overnight in an open dish. Cool. Add 1.2% (w/w) de-ionized H_2O which have been previously extracted 3 times with hexane, to remove traces of organic materials. Store in tightly-capped glass bottle. Shake well and then agitate overnight using a Wheaton Roller. Store 24 hours before use. ***Shelf life:*** 2 months. ***Note:*** It is important to deactivate the Florisil with water, otherwise complete recovery of PCDDs/PCDFs may require large volumes of DCM. Prior to routine use, every new batch of Florisil is tested for the elution of $^{13}C_{12}$-labeled PCDDs/PCDFs and NOPCBs, using method described in 7.7.

4.3. Solutions

4.3.1. DCM/hexane (1:1 v/v)

4.3.2. DCM/hexane (5:95 v/v)

4.4. Stock Standards

4.4.1. PCDDs/PCDFs

4.4.1.1. *Native standards* - PCDDs/PCDFs mixture prepared in nonane solution - Wellington Laboratories EPA-1613PAR. ***Note:*** Contains seventeen congeners (concentrations shown in **Table 1**).

4.4.1.2. *Surrogates* - Isotopically-labeled PCDDs/PCDFs mixture prepared in nonane solution - Wellington Laboratories EPA-1613LCS. ***Note:*** Contains six $^{13}C_{12}$ -labeled PCDDs and nine $^{13}C_{12}$ -labeled PCDFs (concentrations shown in **Table 1**).

4.4.1.3. *Recovery standards and retention time markers* - Isotopically-labeled TCDD/HxCDD - Wellington Laboratories EPA-1613ISS (concentrations shown in **Table 1**).

4.4.1.4. *Window defining mixture* - A mixture (1 ng/µL) containing the earliest and latest eluting PCDDs and PCDFs congeners within each homologous group of congeners (ref. **Table 3** for elution order) - Wellington Laboratories EPADB-5CWDS.

4.4.2. NOPCBs

4.4.2.1. *Native standards* - Cambridge Isotope Laboratories PCB-37; Wellington Laboratories PB-077-S, PB-126-S, PB-169-S, PB-189-S.

4.4.2.2. *Surrogates* - Isotopically labeled NOPCB mixture. Contains three $^{13}C_{12}$ -labeled NOPCBs (PCB-77, PCB-126 and PCB-169). Cambridge Isotope Laboratories EC1404, EC1425 and EC1416 respectively.

4.4.2.3. *Recovery standards and retention time marker* - PCB-112. Ultra-Scientific RPC-070.

4.4.3. Mass spectrometer calibration standard - Perfluorokerosene, high boiling (PFK - BP 210-260°C). Fluka Chemica 77275.

4.5. Working Standards

Note: Refer to SOP-CHEM-PROC-05 for details concerning the preparation and storage of standards solutions.

4.5.1. PCDDs/PCDFs

4.5.1.1. *Native, surrogates and recovery standards and retention time markers* - Prepared by diluting the stock solutions with toluene to produce the concentrations shown in **Table 1**.

4.5.1.2. *Window defining mixture* -A standard (100 pg/µL) is prepared by diluting the stock solutions with toluene.

4.5.1.3. *Five-points calibration standards* - Calibration standards (CS1 through CS5) are prepared in toluene using the standards solution from 4.5.1.1 to produce the concentrations shown in **Table 2**.

4.5.2. NOPCBs

4.5.2.1. *Native standards* - Prepared by mixing and diluting the 5 NOPCBs standards defined in 4.4.2.1 with toluene to produce the concentrations shown in **Table 4.**

4.5.2.2. *Surrogates* - Prepared by mixing and diluting the 3 labeled NOPCBs standards defined in 4.4.2.2 with toluene to produce the concentrations shown in **Table 4.**

4.5.3. *Recovery standard and retention time marker* - PCB 112 (ref. 4.4.2.3) is diluted to a concentration of 200 pg/µL with toluene for the PCDDs/PCDFs (ref. **Table 1**) and to 100 pg/µL for the NOPCBs (ref. **Table 4**)

4.6. QA Reference Material

Herring gull egg homogenate prepared in-house from eggs collected in 1989 from Lake Ontario. ***Note:*** Details on the preparation of this quality assurance material is given in Wakeford 1997 [2.12].

4.7. Method Blank

Chicken eggs free of PCDDs/PCDFs and NOPCBs. ***Note:*** It is usually prepared by collecting the "dump" fraction of the GPC (Section 7.4.3).

TABLE 1 - Composition of PCDDs/PCDFs standard solutions for HRMS

PCDD/PCDFs Standard	Stock Std. (pg/µL)	Working Std. (pg/µL)
Native standards		
2,3,7,8-TCDD	40	20
2,3,7,8-TCDF	40	20
1,2,3,7,8-PeCDD	200	100
1,2,3,7,8-PeCDF	200	100
2,3,4,7,8-PeCDF	200	100
1,2,3,4,7,8-HxCDD	200	100
1,2,3,6,7,8-HxCDD	200	100
1,2,3,7,8,9-HxCDD	200	100
1,2,3,4,7,8-HxCDF	200	100
1,2,3,6,7,8-HxCDF	200	100
1,2,3,7,8,9-HxCDF	200	100
2,3,4,6,7,8-HxCDF	200	100
1,2,3,4,6,7,8-HpCDD	200	100
1,2,3,4,6,7,8-HpCDF	200	100
1,2,3,4,7,8,9-HpCDF	200	100
OCDD	400	200
OCDF	400	200
Surrogates		
$^{13}C_{12}$-2,3,7,8-TCDD	100	50
$^{13}C_{12}$-2,3,7,8-TCDF	100	50
$^{13}C_{12}$-1,2,3,7,8-PeCDD	100	50
$^{13}C_{12}$-1,2,3,7,8-PeCDF	100	50
$^{13}C_{12}$-2,3,4,7,8-PeCDF	100	50
$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	100	50
$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	100	50
$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	100	50
$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	100	50
$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	100	50
$^{13}C_{12}$-2,3,4,6,7,8-HxCDF	100	50
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	100	50
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	100	50
$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	100	50
$^{13}C_{12}$-OCDD	200	100
Recovery standards and retention time marker		
$^{13}C_{12}$-1,2,3,4-TCDD [a]	200	100
$^{13}C_{12}$-1,2,3,7,8,9-HxCDD [b]	200	100
Retention time marker		
PCB-112 [c]	200	200

[a] recovery standard for tetra- and penta-homologues; [b] retention time marker and recovery standard for hexa-, hepta-, and octa-homologues; [c] retention time marker for tetra- and penta- homologues.

TABLE 2 - Composition of PCDDs/PCDFs calibration standards (CS) for HRMS

PCDD/PCDFs Standard	CS1	CS2	CS3 [a]	CS4	CS5
Native standards	(pg/µL)				
2,,3,7,8-TCDD	2.5	5	10	20	40
2,,3,7,8-TCDF	2.5	5	10	20	40
1,2,3,7,8-PeCDD	12.5	25	50	100	200
1,2,3,7,8-PeCDF	12.5	25	50	100	200
2,3,4,7,8-PeCDF	12.5	25	50	100	200
1,2,3,4,7,8-HxCDD	12.5	25	50	100	200
1,2,3,6,7,8-HxCDD	12.5	25	50	100	200
1,2,3,7,8,9-HxCDD	12.5	25	50	100	200
1,2,3,4,7,8-HxCDF	12.5	25	50	100	200
1,2,3,6,7,8-HxCDF	12.5	25	50	100	200
1,2,3,7,8,9-HxCDF	12.5	25	50	100	200
2,3,4,6,7,8-HxCDF	12.5	25	50	100	200
1,2,3,4,6,7,8-HpCDD	12.5	25	50	100	200
1,2,3,4,6,7,8-HpCDF	12.5	25	50	100	200
1,2,3,4,7,8,9-HpCDF	12.5	25	50	100	200
OCDD	25	50	100	200	400
OCDF	25	50	100	200	400
Surrogates					
$^{13}C_{12}$-2,3,7,8-TCDD	50	50	50	50	50
$^{13}C_{12}$-2,3,7,8-TCDF	50	50	50	50	50
$^{13}C_{12}$-1,2,3,7,8-PeCDD	50	50	50	50	50
$^{13}C_{12}$-1,2,3,7,8-PeCDF	50	50	50	50	50
$^{13}C_{12}$-2,3,4,7,8-PeCDF	50	50	50	50	50
$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	50	50	50	50	50
$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	50	50	50	50	50
$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	50	50	50	50	50
$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	50	50	50	50	50
$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	50	50	50	50	50
$^{13}C_{12}$-2,3,4,6,7,8-HxCDF	50	50	50	50	50
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	50	50	50	50	50
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	50	50	50	50	50
$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	50	50	50	50	50
$^{13}C_{12}$-OCDD	100	100	100	100	100
Recovery standards and retention time marker					
$^{13}C_{12}$-1,2,3,4-TCDD [b]	50	50	50	50	50
$^{13}C_{12}$-1,2,3,7,8,9-HxCDD [c]	50	50	50	50	50
Retention time marker					
PCB-112 [d]	100	100	100	100	100

[a] used daily to verify calibration and abundance ratios; [b] recovery standard for tetra- and penta-homologues; [c] retention time marker and recovery standard for hexa-, hepta-, and octa- homologues; [d] retention time marker for tetra- and penta- homologues.

TABLE 3 - Elution order of PCDDs/PCDFs window defining mixture on a 30 m DB5 column

Homologue Group	*First Eluting Isomer*	*Last Eluting Isomer*
TCDD	1,3,6,8-	1,2,8,9-
TCDF	1,3,6,8-	1,2,,8,9-
PeCDD	1,2,4,6,8/ 1,2,4,7,9-	1,2,3,8,9-
PeCDF	1,2,3,6,8/ 1,3,4,6,8-	1,2,3,8,9-
HxCDD	1,2,4,6,7,9/ 1,2,4,6,8,9-	1,2,3,4,6,7-
HxCDF	1,2,3,4,6,8-	1,2,3,4,8,9-
HpCDD	1,2,3,4,6,7,9-	1,2,3,4,6,7,8-
HpCDF	1,2,3,4,6,7,8-	1,2,3,4,7,8,9-
OCDD	-	-
OCDF	-	-

TABLE 4 - Composition of NOPCB standard solutions for HRMS

NOPCBs Standard		Stock Std. (ng/µL)	Working Std. [a] (pg/µL)
Native standards			
	PCB-37	35	100
	PCB-77	200	100
	PCB-126	100	100
	PCB-169	200	100
	PCB-189	200	100
Surrogates			
	$^{13}C_{12}$-PCB-77	40	100
	$^{13}C_{12}$-PCB-126	40	100
	$^{13}C_{12}$-PCB-169	40	100
Recovery standard			
	PCB-112	0.2	100 [b]

[a] used daily to verify calibration and abundance ratios; [b] retention time marker and recovery standard

5. AUXILIARY EQUIPMENT

5.1. Glassware and Labware

5.1.1. Pasteur pipets

5.1.2. Spatulas

5.1.3. Aluminum disposable dishes

5.1.4. Graduated glass pipets, 0.5, 1, 2 and 10 mL

5.1.5. Volumetric glass flasks, 10, 50 and 100 mL

5.1.6. Graduated glass cylinders, 100 mL and 1 L

5.1.7. Glass mortars and pestles

5.1.8. Graduated glass centrifuge tubes, 12 and 15 mL with ℸ13 ground glass stopper

5.1.9. Hamilton™ syringes, 5, 10, 25 and 50 µL

5.1.10. Glass column, 1.0 cm ID x 24 cm long with Teflon™ stopcock and with ℸ19/22 and 24/40 outer joint at top of column (used for extraction, alumina and Florisil clean-up, and lipid determination)

5.1.11. Glass reservoir, 125 mL and 250 mL with ℸ19/22 and 24/40 inner joint for glass column 5.1.10

5.1.12. Glass column, 2.1 cm ID x 35 cm long with Teflon™ stopcock and with ℸ24/40 outer joint at top of column (used for extraction with samples >5 g)

5.1.13. Glass reservoir, 250 mL, with ℸ24/40 inner joint for glass column 5.1.12

5.1.14. Glass column, 3 cm ID x 50 cm long with Teflon™ stopcock and reservoir (for preparing the Na_2SO_4)

5.1.15. Glass wool (Canadawide Scientific 54100-11), pre-washed with DCM/hexane (1:1) and air dried

5.1.16. Flat bottom flasks, 125, 250 and 500 mL all with ℸ24/40 outer joint

5.1.17. Reactivials, 100 µL, 2 mL and 4 mL with Mininert™ valve (Chromatographic Specialties Inc.)

5.1.18. Amber glass vials with cap and Teflon™ seal (red) 8 mm - for autoinjector (Chromatographic Specialties Inc. C37088 - vials and C220850 - caps)

5.1.19. GPC glass column, 3 cm ID x 60 cm long - Envirosep-ABC column assay (ABC Laboratories Inc., Columbia, MO, USA)

5.1.20. Glass column for carbon/glass fibre, 6.5 mm ID x 10 cm long (Omnifit)

5.1.21. Flanged Pyrex™ column with variable and fixed end-piece (Anspec, Ann Arbor, MI, USA)

5.1.22. Glass funnel, 25 mm and 10 cm

5.1.23. Graduated Pyrex™ centrifuge tube, 15 mL

5.1.24. Glass-fibre paper, Whatman GFD-3 (Whatman International Ltd.)

5.1.25. Hand crimper, 8 mm - for crimping aluminum seals to autosampler vials (Chromatographic Specialties)

5.1.26. Glass syringe, 10 mL (B-D D3037)

5.1.27. C18 cartridge (Superclean ENVI-18, 6 mL tubes) Supelco 505706

5.1.28. Scintillation vials, 10 mL with caps

5.1.29. Scissors

5.1.30. Aluminum foil, hexane rinsed

5.1.31. Amber glass jar (500 mL) with cap

5.2. Equipment

5.2.1. Analytical and top-loading balance

5.2.2. Vortex mixer

5.2.3. Rotary evaporator with water bath (Buchi 461, Brinkman Instruments)

5.2.4. Refrigerated circulating bath at ca -15°C

5.2.5. Nitrogen evaporator (adjusted at low setting) to give temperature of ca 35°C

5.2.6. Drying oven (Fisher Scientific, Model 516 G)

5.2.7. Muffle furnace (Blue M Electric Company, Blue Island, Il, USA)

5.2.8. Visiprep solid phase extraction vacuum manifold (Supelco 57030) with Visidry drying attachment (Supelco 57100)

5.2.9. Homogenizer (Polytron PT-10, Brinkman Instruments)

5.3. Instrumentation

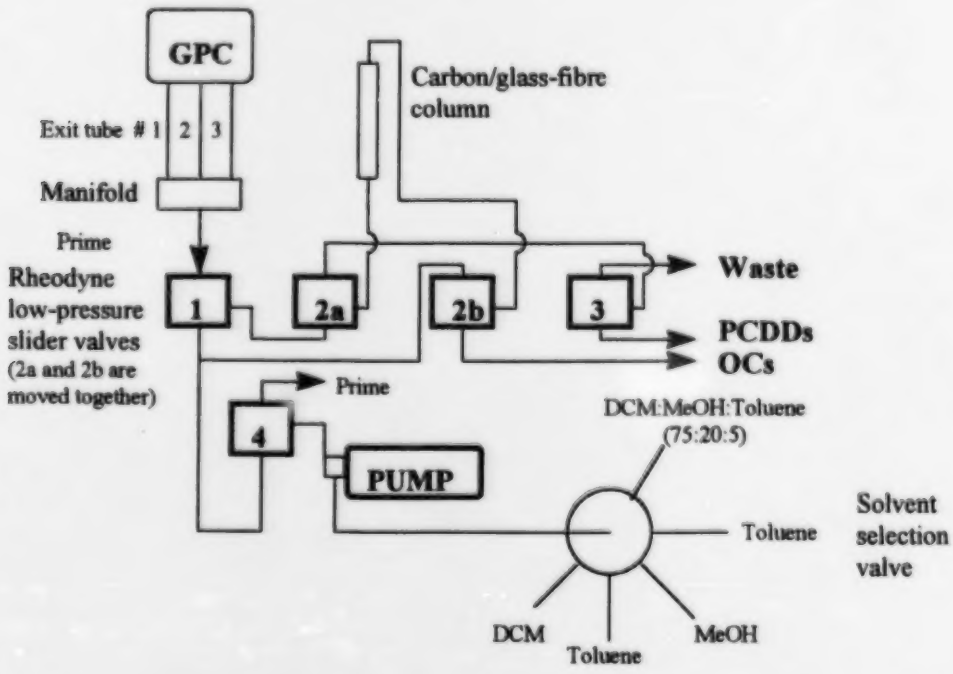
5.3.1. Automated gel-permeation chromatograph GPC Autoprep 1002A (Analytical Biochemistry Labs Inc., Columbia, MO, USA), with 23 sample loops (5 mL volume).

5.3.2. Automated GPC/carbon chromatograph (customized at NWRC for the purpose of fully automated sample loading onto the carbon column, followed by forward and reverse elution and regeneration of the carbon

column, with a choice of up to 4 solvents). It is controlled by a Chromat-A-Trol Model II (Eldex) controller, and consist of a low or high pressure solvent pump (Eldex Model E-120-S) capable of delivering ca 5 mL/min, a six port selection valve (Rheodyne), 5 three-way low-pressure slider valves, a 5 mL Teflon™ sample loop (1.5 mm OD, 0.8 mm ID) with standard connectors (1/4 x 28 thread, Supelco), and the carbon/glass fibre column (5.1.20). See diagram in **Figure 1**. ***Note:*** Only one column is illustrated but the apparatus is set up to run 3 carbon columns simultaneously.

5.3.3. HRGC/HRMS, Hewlett-Packard gas chromatograph (GC) 5890 Series II equipped with a Carlo Erba CTC-A200S autosampler and linked to a VG AutoSpec Double-focusing high resolution mass spectrometer (MS), with a DKA-300 VAX 4000 computer equipped with OPUS software Version 1.7 (including "Traces" and "Dioxin" programs for peak processing and quantitation). GC column: 30 m DB-5 (J&W) fused silica column, 0.25 mm ID, 0.25 µm film thickness (Chromatographic Specialties J1225032).

FIGURE 1 - GPC/carbon column chromatography apparatus



6. SPECIMEN OR SAMPLE HANDLING REQUIREMENTS

Samples provided to the Trace Organic Chemistry Laboratory are prepared as described in the Tissue Preparation Unit's standard operating procedure SOP-TP-PROC-07. These tissues were usually collected and preserved as recommended in the document "Protocol for Field Collection and Storage of Wild Birds for Biomarker Studies" (S. Trudeau, Biomarker Laboratory, NWRC, 1992).

7. PROCEDURE

Flow chart is given in **Figure 2.**

7.1. Columns Preparation

7.1.1. *GPC Column*

Pack GPC column (Section 5.1.19) with 60 g Envirobeads ™ S-X3 pre-swelled (equilibrated) in DCM/hexane (1:1 v/v) mobile phase. ***Note:*** This material generally makes 43 to 45 cm in column length.

7.1.2. *Carbon Column*

7.1.2.1. Heat AX-21 carbon at 105°C for 2 hours. Let it cool to room temperature and transfer in a tightly capped glass jar.

7.1.2.2. Cut 1.5 g of Whatman glass-fibre paper (Section 5.1.24) with scissors into 0.3 - 0.5 cm pieces. Put into a 250 mL beaker, add 150 mL DCM and homogenize with Polytron. ***Note***: This constitutes packing material for 3 columns.

7.1.2.3. Add 150 mg activated carbon (Section 7.1.2.1) to the glass-fibre/DCM suspension, mix gently with Polytron and divide into 3 equal portions.

7.1.2.4. Pack the suspension into the carbon/glass-fibre column (Section 5.1.20) using a small glass funnel. Compress packing in each column to a 6 cm length.

7.1.2.5. Connect the columns to the automated carbon chromatography apparatus and condition the columns by running a complete "regeneration" cycle, then a "run" cycle (see Section 7.6).

7.1.2.6. Verify the new columns by testing the recovery of $^{13}C_{12}$-labeled PCDDs/PCDFs and NOPCBs as described in Section 7.6.

7.1.3. *Alumina column*

Prepare fresh daily as described in Sections 7.5.1.-7.5.2.

7.1.4. *Florisil column*

Prepare fresh daily as described in sections 7.7.1.-7.7.2.

7.2. Extraction - Tissue samples

Note: For plasma see Section 7.3.

7.2.1. Grind 5.00 g of the sample with 30 g of the treated anhydrous Na_2SO_4 (Section 4.1.7) in a glass mortar and pestle until a free-flowing mixture is obtained.

7.2.2. Plug a 1 cm ID x 24 cm long glass column with some treated glass wool (Section 5.1.15), add about 1 cm Na_2SO_4 at the bottom and half fill the column with hexane. (***Note:*** column described in Section 5.1.12 is used for samples >5 g). Pour ground sample mixture into the glass column and tap the column gently to settle the mixture. Rinse the mortar and pestle with DCM/hexane (1:1), and transfer the rinse onto the top of the column using a Pasteur pipet, repeat rinsing mortar and pestle three times.

7.2.3. Place a 250 mL flat bottom evaporating flask under the column. Allow DCM/hexane to drain to surface of packing.

7.2.4. Spike the top of the column with 10 µL of $^{13}C_{12}$-labeled PCDDs/PCDFs surrogates (50 pg/µL tetra to hepta and 100 pg/µL octa - ref. **Table 1**), and with 10 µL of $^{13}C_{12}$-NOPCBs surrogates (100 pg/µL each - ref. **Table 4).** Pipet 3 x 1 mL hexane on top of the column and drain to surface of packing in between.

7.2.5. Elute the column with 150 mL DCM/hexane (1:1) at 5-10 mL/min, and collect the eluent.

7.2.6. Evaporate the eluent to less than 2 mL on a rotary evaporator with water bath at ca 30°C, then quantitatively transfer into a 12 mL

graduated centrifuge tube. Adjust the final volume to 3 mL with DCM/hexane (1:1). The lipid extract is now ready for GPC cleanup (Section 7.4.2).

7.3. **Extraction** - Plasma

7.3.1. Spike a 5 mL aliquot of the thawed plasma (accurately weighed in a 15 mL graduated centrifuge tube) with 10 µL of $^{13}C_{12}$-labeled PCDDs/PCDFs surrogates (50 pg/µL tetra to hepta and 100 pg/µL octa - ref. **Table 1**), and with 10 µl of $^{13}C_{12}$-NOPCBs surrogates (100 pg/µL each - ref. **Table 4**). Mix the spiked plasma gently with a Vortex mixer, and let it stand for 30 min to equilibrate.

7.3.2. Add 5 mL formic acid (1:1) to the spiked plasma in order to denature proteins, mix gently with Vortex mixer, and let it stand for 15 min.

7.3.3. Activate C18 cartridge with two 6 mL portions of methanol followed by two 6 mL portions of de-ionized water, using the Visiprep solid phase vacuum manifold (Section 5.2.8).

7.3.4. Load the sample mixture (from 7.3.2) onto the activated C18 cartridge with suction at a flow rate of 6-7 mL/min. The polar interferences and lipids are not retained by the cartridge.

7.3.5. Dry the C18 cartridge thoroughly with a stream of nitrogen gas using a Visidry Drying attachment for ca 35 min. ***Note:*** Incomplete drying of the cartridge would result in sample loss.

7.3.6. Elute the analytes from the dried C18 cartridge 3 x with 2 mL of DCM/hexane (1:1). The sample is now ready for alumina column cleanup (Section 7.5).

7.4. **Gel Permeation Chromatography (GPC)**

7.4.1. Before routine analysis is performed, verify the "dump" (reject) volume and the "collect" volume of the Envirobeads™ S-X3 column by running a standard solution, such as 10 µL $^{13}C_{12}$-labeled PCDDs/PCDFs surrogates on the GPC, and collecting and analyzing 20 mL fractions of the eluent. Lipids elute in the "dump" fraction, OCs (including PCDDs, PCDFs and NOPCBs) elute in the "collect" fraction. The "dump" volume is 140 mL and the "collect" volume is 160 mL.

7.4.2. Quantitatively transfer lipid extract from 7.2.6 into a GPC loop (<1 g lipid/loop). Each loop holds exactly 5 mL and there is a dead volume of ca 1.5 mL between the injector valve and the sample loop. Quantitative transfer requires the injection of the 3 mL sample into the loop using a 10 mL glass syringe, followed by rinsing the tube with 3 x 1 mL portions of DCM/hexane (1:1), injecting each washes into the loop. ***Note:*** Samples with high lipid content can be split in 2 or 3 loops and then combined.

7.4.3. Set GPC flow-rate at 5 mL/min of DCM/hexane (1:1) and initiate the operation of the GPC. The GPC automatically directs the "dump" cycles for each run to a common waste container, and each of the "collect" cycles sequentially to a numbered exit tube corresponding to the respective sample loop placed into a 250 mL flat bottom evaporating flask. ***Note:*** It is possible to load and run as many as 23 samples simultaneously. The sequence can be run overnight.

7.4.4. Evaporate eluent from 7.4.3 to less than 2 mL on a rotary evaporator with water bath temperature at ca 35°C. The sample is now ready for alumina column cleanup.

7.5. Alumina Column Cleanup

7.5.1. Prepare alumina column by adding 10 g basic alumina (Section 4.2.1) to a 1 cm ID glass column (Section 5.1.10) half filled with hexane. Add 1 cm of the treated anhydrous sodium sulphate (Section 4.1.7) onto the top of the column.

7.5.2. Tap the column gently, allow hexane to drain to surface of packing, and place a 125 mL flat bottom evaporating flask under the column.

7.5.3. Quantitatively load sample extract (from Section 7.4.4 or 7.3.6) on top of the column using Pasteur pipet. Rinse the evaporating flask 3-4 times with small portions of hexane, transfer all rinses on top of the column allowing solvent to drain to packing level in between rinses.

7.5.4. Place glass reservoir (Section 5.1.11) on the column and elute with 80 mL DCM/hexane (1:1) at 5 mL/min.

7.5.5. Evaporate the eluent almost to dryness with rotary evaporator, water bath temperature at ca 30°C. ***Caution:*** Never take the sample

completely to dryness, to avoid problems with recoveries (OCDD may adsorb to the glass or trichloro - and tetrachloro NOPCBs may evaporate).

7.5.6. Add 5 mL hexane and re-evaporate to less than 2 mL. ***Caution:*** the sample has to be free of DCM, in order to prevent losses of NOPCBs at the carbon column cleanup.

7.5.7. Quantitatively transfer the sample with hexane into a 12 mL graduated centrifuge tube and adjust the final volume to 3 mL with hexane. The sample is now ready for carbon column chromatography.

7.6. Carbon/Glass Fibre Column Separation

This procedure is designed to separate PCDDs/PCDFs and NOPCBs from other OC compounds, using the automated carbon column apparatus described in 5.3.2. ***Note:*** A sequence of 3 samples can be run overnight.

7.6.1. Regenerate the column prepared in 7.1.2. sequentially with 50 mL toluene, 50 mL methanol, 50 mL toluene, 50 mL DCM and 50 mL hexane.

7.6.2. Put the injector valve on the carbon column apparatus in "load" mode manually. Load the 3 mL sample extract from 7.5.7 quantitatively into the sample loop using a 10 mL glass syringe. Each loop holds exactly 5 mL and there is a dead volume of ca 0.1 mL between the injector valve and the sample loop. Quantitative transfer requires the injection of the 3 mL sample extract, followed by rinsing the centrifuge tube 3 times with 0.5 mL portions of hexane, and injecting each washes into the loop.

7.6.3. Put the injector valve manually in "run" mode and immediately initiate the operation of the carbon chromatography apparatus, so it executes the pre-set sequence as follows: **a)** loads sample extract from sample loop into the carbon/glass-fibre column with 40 mL hexane, **b)** elute the column with 180 mL DCM in the opposite direction. These fractions are collected together in a 500 mL flask (they contain all the non-aromatic and most of the aromatic OC compounds from the sample, including PCBs except NOPCBs, PCDDs/PCDFs) and **c)** PCDDs /PCDFs and NOPCBs are back eluted from the carbon column with a reverse flow of 180 mL toluene, into a 250 mL flask.

7.6.4. Evaporate OC fraction to 5 mL with rotary evaporator, water bath temperature adjusted to ca 30°C. Quantitatively transfer into a 10 mL scintillation vial and store in the dark, at room temperature, for future use.

7.6.5. Evaporate the toluene fraction (PCDDs/PCDFs/NOPCBs) almost to dryness with rotary evaporator, water bath temperature at ca 50°C. ***Caution:*** never take the sample completely to dryness, because OCDD may absorb to the glass and trichloro- tetrachloro-NOPCBs may evaporate.

7.6.6. Add 5 mL hexane and re-evaporate to less than 2 mL. The sample extract is now ready for Florisil column separation.

7.7. Florisil Column Separation

Florisil column cleanup is designed to separate NOPCBs from PCDDs/PCDFs.

7.7.1. Prepare Florisil column by adding 8 g of the de-activated Florisil (Section 4.2.2) into a 1 cm ID glass column (Section 5.1.10) half filled with hexane. Add 1 cm of treated anhydrous sodium sulphate to the top of the column.

7.7.2. Tap the column gently, allow hexane to drain to surface of packing, and place a 125 mL flat bottom evaporating flask under the column.

7.7.3. Quantitatively load sample from 7.6.6 on top of the Florisil column using Pasteur pipet. Rinse the flask 3-4 times with small portions of hexane. Transfer all rinses on top of the column, allowing solvent to drain to packing level in between rinses.

7.7.4. Place glass reservoir (Section 5.1.11) on the column and elute with 50 mL DCM/hexane (5:95), at 2-3 mL/min. This fraction contains native and labeled NOPCBs.

7.7.5. Place a new 250 mL flat bottom evaporating flask under the column, and elute the column with 150 mL DCM at 5 mL/min. This fraction contains native and labeled PCDDs/PCDFs.

7.7.6. Evaporate both fractions (from 7.7.4 and 7.7.5) to less than 2 mL each, with rotary evaporator, water bath temperature at ca 30°C, and

quantitatively transfer (with hexane) into graduated 12 mL glass centrifuge tubes.

7.7.7. Further evaporate each fraction with a gentle stream of purified nitrogen to 1 mL, add ca 0.1 mL toluene as a "keeper" to each, then reduce volumes to 0.1 mL.

7.7.7.1. *NOPCBs fraction:*

Using a 10 µL Hamilton syringe add exactly 5 µL (200 pg/µL) PCB-112 performance internal (recovery) standard/retention time marker into a 100 µL autosampler vial which has been previously marked at the 10 µL level. Transfer the clean NOPCB extract from 7.7.7 to the autosampler vial with a Pasteur pipet and rinse the centrifuge tube with 2 x 2 drops of toluene and transfer to the autosampler vial.

Reduce the final volume to the 10 µL mark with a gentle stream of purified nitrogen. (***Note:*** Exact volume is not critical, since internal standard quantitation is used to determine residue levels). Retain for HRGC/HRMS analysis of native and labeled NOPCBs.

7.7.7.2. *PCDDs/PCDFs fraction:*

Using a 10 µL Hamilton syringe add exactly 5 µL $^{13}C_{12}$-labeled tetra/hexa-PCDDs (100 pg/µL each) as a performance internal (recovery) standard (**Table 1**) and 5 µL PCB-112 (200 pg/µL) retention time marker standard into a 100 µL autosampler vial which has been previously marked at the 10 µL level. Transfer the clean PCDDs/PCDFs extract from 7.7.7 to the autosampler vial with a Pasteur pipet and rinse the centrifuge tube with 2 x 2 drops of toluene and add to the autosampler vial.

Reduce the final volume to the 10 µL mark with a gentle stream of purified nitrogen. (***Note:*** Exact volume is not critical, since internal standard quantitation is used to determine residue levels). Retain for HRGC/HRMS analysis of native and labeled PCDDs/PCDFs.

7.8. HRGC Operating Conditions

7.8.1. *Column*

- 30 m long DB-5 fused-silica column, 0.25 mm ID, 0.25 μm film thickness

7.8.2. *Injection information*

- Injection port temperature 260°C
- Splitless injection
- Sample washes 0
- Solvent washes 20
- Pull-up count 10
- Sample volume 1 μL
- Air volume 0.5 μL
- Filling volume 3 μL
- Pull-up delay 0.5 s
- Pre-inj. delay 1.0 s
- Post-inj. delay 1.5 s

7.8.3. *Oven temperature programme*

- 100°C, hold 3 min; 20°C /min to 180°C; 5°C /min to 325°C

7.8.4. *Carrier gas (He)*

- Head pressure 1.1 kg/cm^2 (45 cm/sec at 100°C)

7.8.5. *Chromatographic windows*

- for TCDD/Fs 15.5 - 20.5 min
  PCDD/Fs 20.5 - 23.3 min
  HxCDD/Fs 23.3 - 26.5 min
  HpCDD/Fs 26.5 - 29.5 min
  OCDD/F 29.5 - 32.5 min

- for PCBs 37,77,81 13.5 - 19.0 min
  PCBs 126,169,189 19.2 - 27.5 min

7.9. HRMS Operating Conditions

7.9.1. *HRMS conditions*

- Ionising electron energy 34 eV
- SIR voltage mode selected ion monitoring mode
- Dwell time (on each ion) 50 ms (for PCDDs/PCDFs)
  30 ms (for NOPCBs)
  50 ms (for PFK calibration standard)
- Source temperature 280°C
- Transfer line temperature 280°C
- Calibration standard PFK T° 170°C

7.9.2. *Masses selection for PCDDs/PCDFs*

(min)	*15.5-20.5*	*20.5-23.3*	*23.3-26.5*	*26.5-29.5*	*29.5-32.5*
(m/z)	303.9016	339.8597	373.8207	407.7818	441.7428
	305.8987	341.8568	375.8178	409.7788	443.7398
	315.9419	351.9000	380.976 [a]	419.8220	454.9728 [a]
	316.9824 [a]	353.8970	383.8639	421.8291	457.7377
	317.9389	355.8546	385.8610	423.7767	459.7348
	319.8965	357.8517	389.8156	425.7737	469.7780
	321.8936	366.9792 [a]	391.8127	430.9728 [a]	471.7750
	325.8800	367.8949	401.8559	435.8169	513.6775
	327.8770	369.8919	403.8530	437.8140	
	331.9368	409.7974	445.7555	479.7165	
	333.9339				
	339.8597				
	375.8364				

[a] PFK calibration ion

Note:

- The two strongest ions in the molecular cluster are monitored in every retention time windows for each native and labeled PCDDs/PCDFs.
- Ion 339.8597 is monitored in the first retention time window as well as in the second window because, in some tissue samples, the first eluting pentachloro-furan (12389-P5CDF) is detected, and this congener elutes in the tetrachloro-retention time window, near to the last eluting tetrachloro-dioxin (1368-T4CDF).

- When the 12389-P5CDF is present, levels are calculated manually, by comparing the area of 12389-P5CDF to areas and levels of other P5CDFs detected in the P5CDF-chromatographic window.
- One mass is monitored in every window for chlorinated diphenyl ether (with one more chlorine than the PCDD has in the same window) which may interfere.
- Mass 327.8770 in the first window is to measure PCB-112, which is used as a retention time marker standard (see target ions in **Table 8**).

7.9.3. *Masses selection for NOPCBs*

(min)	*13.5-19.0*	*19.0-27.5*
(m/z)	255.9610	325.8800
	257.9580	327.8770
	289.9220	337.9210
	291.9190	339.9180
	292.9824 [a]	342.9792 [a]
	301.9630	359.8410
	303.9600	361.8390
	325.8801	371.8820
	327.8770	373.8790
		393.8020
		395.8000

[a] PFK calibration ion

Note :

- The two strongest ions in the molecular cluster are monitored in each retention time windows for each native and labeled NOPCBs.
- Two masses are monitored in the first window for PCB-112, which is used as a retention time marker/recovery standard (see target ions in **Table 8**).

7.10. HRMS Calibration

Note: For detailed instructions on the operation of the instruments, consult the equipment operator's manuals [2.9].

7.10.1. Tune the HRMS daily (prior to sample acquisition) with a PFK calibration standard as described in SOP-CHEM-PROC-11. ***Note:*** PCDDs/PCDFs analysis requires a resolution of 10 000 (5% valley) NOPCBs analysis requires a resolution of 7 000 (5 % valley).

7.10.2. Print hard copies of the tuning data for each chromatographic window

and archive them along with the sample chromatograms. An example of the peaks obtained is given in **Figure 3**.

7.11. Instrument Daily Calibration Verification

Note: Initial calibration with CS1 to CS5 is detailed in Section 7.13.

7.11.1. Establish the operating conditions given in Sections 7.8 and 7.9.

7.11.2. Inject 1 µL of the PCDDs/PCDFs daily calibration standard CS3 (**Table 2**).

7.11.3. Start the GC column initial isothermal hold upon injection and collect data as defined in the chromatographic windows table (Section 7.8.5).

7.11.4. Enter sample information, ions, chromatographic windows and integration parameters on the "Traces" (Section 5.3.3) spreadsheet.

7.11.5. Run the "Traces" peak processing program to integrate peaks for each selected ions, and print chromatograms and results.

7.11.6. Retrieve the "Target" table from the most recent CS3 analyzed.

7.11.7. Run the "Dioxin" (Section 5.3.3) quantitation program to calculate Relative Response Factors (RRF - response factor of unlabeled relative to the $^{13}C_{12}$-labeled internal standard), for each PCDD/PCDF congener (see example in **Table 5,** and details on quantitation in Section 8.1). *Note:* The calculated concentration for each native congener must be within 20 % of its known value. Performance criteria are detailed in Section 10.

7.11.8. Generate a one-point calibration curve using the "Dioxin" quantitation program (using data from 7.11.7). See example in **Table 6.**

7.11.9. Print hard copy of the table for the one point calibration curve (generated in Section 7.11.8).

7.11.10. Store computerized data and hard-copies as described in SOP-CHEM-PROC-08.

7.11.11. Repeat steps 7.11.2 to 7.11.9 with the NOPCBs standard working

solution (**Table 4**) using the "Target" table created for NOPCBs. If the performance criteria are met, proceed with the analysis of the samples.

7.12. HRGC/HRMS Samples Analysis

7.12.1. Analyze the concentrated extract (from Section 7.7.7.1 for NOPCB fraction or 7.7.7.2. for PCDDs/PCDFs fraction) as described for the daily calibration standards (steps 7.11.2 to 7.11.5).

7.12.2. Retrieve the "Target" table from 7.11.6, and the RRF values obtained in Section 7.11.7. Run the "Dioxin" quantitation program to calculate the residue levels and minimum detectable levels for each congener, using *Isotope dilution quantitation* method. Quantitation details are given in Section 8.2.

7.12.3. Print hard copy of the final result table (see example of results in **Table 7**).

7.12.4. Store computerized data and hard-copies as described in SOP-CHEM-PROC-08.

7.13. Five-point Calibration Curve

7.13.1. To calibrate the analytical system and determine linearity, inject sequentially, 1µL of the PCDDs/PCDFs calibration standard mixtures - CS1, CS2, CS3, CS4 and CS5 (Section 4.5.1.3 and **Table 2**). Repeat every 6 months (or whenever new calibration standard solutions are prepared), with the instruments conditions listed in 7.8. and 7.9.

7.13.2. Integrate peaks for each selected ions, print chromatograms and results, using the "Traces" peak processing program (Section 5.3.3).

7.13.3. Generate and print a "Target" table for each of the five calibration standard mixtures using the "Dioxin" quantitation program (Section 5.3.3), (see example **Table 8**).

7.13.4. Run the "Dioxin" quantitation program (for each of the 5 injection) to calculate Relative Response Factors (RRF - response factor of unlabeled relative to the $^{13}C_{12}$-labeled internal standard) for each PCDD/PCDF congener. Generate a five-point calibration curve using the " Dioxin" quantitation program.

7.13.5. Print hard copy of the table for the five-point calibration curve (example given in **Table 9)**.

7.13.6. Store computerized data and hard-copies as per SOP-CHEM-PROC-08.

7.14. Moisture Determination

7.14.1. Put approximately 1 g of sample (tissue homogenate) into a pre-weighed aluminum dish and record the weight to 4 decimal places.

7.14.2. Place the dish in a drying oven at 105°C for about two hours, until constant weight is obtained.

7.14.3. The calculation of the moisture content is as follows:

$$\% \text{ moisture} = 100 - (Wd/Ww) \times 100$$

where: Wd = weight of dry sample
Ww = weight of wet sample

7.15. Lipid Determination

Note: Lipid levels are determined to allow calculations based on lipid content instead of wet-weight, if desired. If lipid determinations in blood plasma is required, consult CWS Technical Report No. 335 "Multiresidue Methods for the Determination of Chlorinated Pesticides and Polychlorinated Biphenyls (PCBs) in Wildlife Tissues". For tissues other than blood plasma, proceed as follows:

7.15.1. Grind a 1 g (accurately weighed) sample aliquot with 15 g of anhydrous Na_2SO_4 in a glass mortar and pestle until a free-flowing mixture is obtained.

7.15.2. Pack the dry sample/Na_2SO_4 mixture with DCM/hexane (1:1) into a 1 cm ID x 24 cm long glass column (5.1.10) which has been plugged with glass wool, and half filled with DCM/hexane (1:1). Rinse the mortar and pestle with DCM/hexane (1:1, v/v), transfer the rinse on top of the column using a Pasteur pipet. Repeat rinsing 3 times.

7.15.3. Tap the column gently to settle the mixture, place a 125 mL flat bottom evaporating flask under the column, and elute lipids with 60 mL

DCM/hexane (1:1) at 3 mL/min.

7.15.4. Concentrate the lipid extract to less than 2 mL using the Rotavapor with the water bath adjusted to ca 30°C.

7.15.5. Quantitatively transfer lipid extract into a pre-weighed aluminum dish using a Pasteur pipet.

7.15.6. Evaporate to dryness at room temperature in the fume hood.

7.15.7. Heat dish in oven at 105°C for 20 to 30 min.

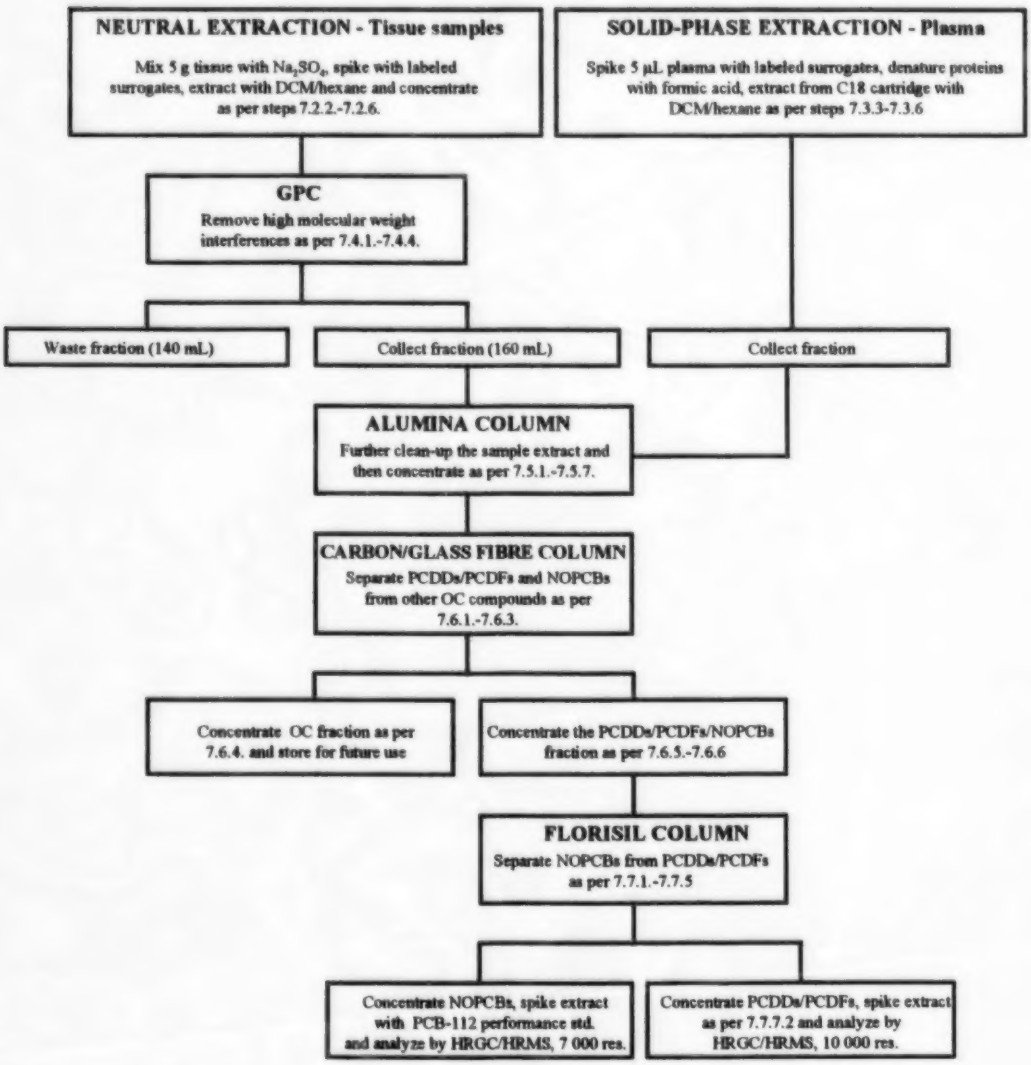
7.15.8. Take dish out of the oven, allow to cool and reweigh. The difference in weight is the weight of lipid in the sample.

7.15.9. The calculation of the lipid content is as follows:

$$\% \text{ lipid} = (Wl \times 100) / Wte$$

where: Wl = weight of lipid
Wte = weight of sample extracted

FIGURE 2 - Flow diagram of extraction, clean up and analysis of PCDDs/PCDFs and NOPCBs


NEUTRAL EXTRACTION - Tissue samples
Mix 5 g tissue with Na_2SO_4, spike with labeled surrogates, extract with DCM/hexane and concentrate as per steps 7.2.2.-7.2.6.
SOLID-PHASE EXTRACTION - Plasma
Spike 5 µL plasma with labeled surrogates, denature proteins with formic acid, extract from C18 cartridge with DCM/hexane as per steps 7.3.3-7.3.6
GPC
Remove high molecular weight interferences as per 7.4.1.-7.4.4.
Waste fraction (140 mL)
Collect fraction (160 mL)
Collect fraction
ALUMINA COLUMN
Further clean-up the sample extract and then concentrate as per 7.5.1.-7.5.7.
CARBON/GLASS FIBRE COLUMN
Separate PCDDs/PCDFs and NOPCBs from other OC compounds as per 7.6.1.-7.6.3.
Concentrate OC fraction as per 7.6.4. and store for future use
Concentrate the PCDDs/PCDFs/NOPCBs fraction as per 7.6.5.-7.6.6
FLORISIL COLUMN
Separate NOPCBs from PCDDs/PCDFs as per 7.7.1.-7.7.5
Concentrate NOPCBs, spike extract with PCB-112 performance std. and analyze by HRGC/HRMS, 7 000 res.
Concentrate PCDDs/PCDFs, spike extract as per 7.7.7.2 and analyze by HRGC/HRMS, 10 000 res.

8. EXPRESSION OF RESULTS

8.1. Calculation of Relative Response Factor (RRF)

An RRF is the ratio of analyte response factor to the response factor of the corresponding labeled surrogate.

Native and $^{13}C_{12}$ labeled standards are analyzed daily prior to sample analysis (ref. Section 7.11.7) and RRF values are determined as follows:

$$RRF = [(A1_n + A2_n) \times C_l] / [(A1_l + A2_l) \times C_n]$$

where: $(A1_n + A2_n)$ = the areas of the two strongest ions (m/z) in the molecular ion cluster for the native CDD/CDF compound in the standard solution

$(A1_l + A2_l)$ = the areas of the two strongest ions (m/z) in the molecular ion cluster for the labeled CCD/CDF compound in the standard solution

C_l = the concentration of the labeled compound in the calibration standard

C_n = the concentration of the native compound in the calibration standard

8.2. Calculation of Analyte Concentration

Isotope dilution quantitation - By adding a known amount of labeled compounds (surrogates) to every sample prior to extraction (Section 7.2.4 and 7.3.1), correction for recovery of the PCDDs/PCDFs/NOPCBs is made, because the native and their labeled analogs exhibit similar effects upon extraction, concentration, and gas chromatography [2.11]. Using the surrogate responses from the sample run, and the RRF values (Section 8.1), recovery corrected concentrations of PCDDs/PCDFs/NOPCBs is calculated directly.

Calculation is done as follows:

$$C_{ex}\,(ng/kg) = [(A1ex_n + A2ex_n) \times Cs_l] / [(A1ex_l + A2ex_l) \times RRF]$$

where: Cex = the concentration of the native CDD/CDF in the extract

$(A1ex_n + A2ex_n)$ = the areas of the two strongest ions (m/z) in the molecular ion cluster for the native CDD/CDF surrogate compound in the sample extract

$(A1ex_l + A2ex_l)$ = the areas of the two strongest ions (m/z) in the molecular ion cluster for the labeled CCD/CDF surrogate compound in the samples extract

Cs_l = the concentration of the labeled compound in the sample extract

RRF = relative response factor, response factor of unlabeled relative to the $^{13}C_{12}$-labeled internal standard

Note: The "Dioxin" software calculates a minimum detection limit for each analyte using a pre-set algorithm.

8.3. Calculation of Recovery for $^{13}C_{12}$ Surrogate Standards

Recoveries (%R) are calculated and reported, as these values indicate the overall quality of the residue data.

Formula comparing the areas in two separate GC injections:

$$\%R = [(A1ex_l + A2ex_l) \times (A1_n + A2_n) \times 100] / [(A1_l + A2_l) \times (A1ex_n + A2ex_n)]$$

where: $(A1ex_l + A2ex_l)$ = the areas of the two strongest ions (m/z) in the molecular ion cluster for the labeled CDD/CDF compound in the sample extract

$(A1_l + A2_l)$ = the areas of the two strongest ions (m/z) in the molecular ion cluster for the labeled CCD/CDF compound in the samples extract

$(A1_n + A2_n)$ = the areas of the two strongest ions (m/z) in the molecular cluster of the performance (recovery) internal standard (Section 7.7.7.2) in the standard injection

$(A1ex_n + A2ex_n)$ = the areas of the two strongest ions (m/z) in the molecular cluster of the performance (recovery) internal standard (Section 7.7.7.2) in the sample injection

9. REPRESENTATIVE DOCUMENTS

FIGURE 3 - Tuning data for PCDDs/PCDFs

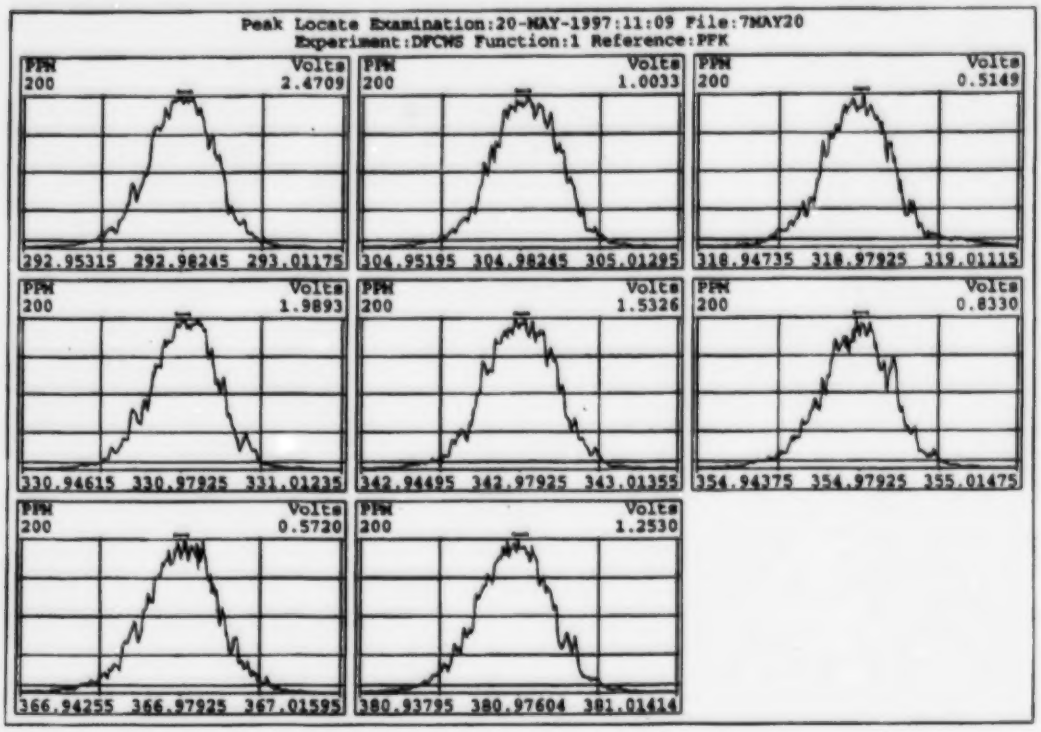


TABLE 5 - Internal Calibration Result

Weight :1 Name	Total Response	Isotope Ratio		R. mm:	T. ss		RRF	pg	Rec/ MDL
PCB-112	109335000	1.58	Y	14:	11	Y	0.00	200.00	
13C-1,2,3,4-TCDD	30446000	0.78	Y	17:	3	Y	1.00	100.00	
13C-2,3,7,8-TCDF	47599000	0.76	Y	16:	58	Y	1.56	100.00	
2,3,7,8-TCDF	8954700	0.7	Y	16:	59	Y	0.94	20.00	0.182
Total TCDF							0.94		
13C-2,3,7,8-TCDD	28388000	0.79	Y	17:	21	Y	0.93	100.00	
2,3,7,8-TCDD	5842000	0.65	Y	17:	22	Y	1.03	20.00	0.550
Total TCDD							1.03		
13C-1,2,3,7,8-PeCDF	35775000	1.65	Y	19:	21	Y	1.18	100.00	
1,2,3,7,8-PeCDF	43796000	1.58	Y	19:	22	Y	1.22	100.00	0.060
2,3,4,7,8-PeCDF	44691000	1.55	Y	20:	2	Y	1.25	100.00	0.059
Total PECDF							1.24		
13C-1,2,3,7,8-PeCDD	21523900	1.72	Y	20:	15	Y	0.71	100.00	
1,2,3,7,8-PeCDD	23893700	1.63	Y	20:	16	Y	1.11	100.00	0.326
Total PECDD							1.11		
13C-1,2,3,7,8,9-HxCDD	29435000	1.3	Y	23:	10	Y	0.00	100.00	
13C-1,2,3,4,7,8-HxCDF	29726100	0.5	Y	22:	9	y	1.01	100.00	
1,2,3,4,7,8-HxCDF	39492000	1.27	Y	22:	10	Y	1.33	100.00	0.071
1,2,3,6,7,8-HxCDF	49595000	1.3	Y	22:	16	Y	1.67	100.00	0.057
1,2,3,7,8,9-HxCDF	40850000	1.25	Y	22:	45	Y	1.37	100.00	0.069
2,3,4,6,7,8-HxCDF	33734000	1.32	Y	23:	25	Y	1.13	100.00	0.083
Total HXCDF							1.38	100.00	0.084
13C-1,2,3,6,7,8-HxCDD	28437000	1.28	Y	22:	58	Y	0.97	100.00	
1,2,3,4,7,8-HxCDD	21944300	1.3	Y	22:	54	Y	0.77	100.00	0.028
1,2,3,6,7,8-HxCDD	29105000	1.33	Y	22:	58	Y	1.02	100.00	0.021
1,2,3,7,8,9-HxCDD	26073000	1.29	Y	23:	11	Y	0.92	100.00	0.023
Total HXCDD							0.90		
13C-1,2,3,4,6,7,8-HpCDF	32327000	1.01	Y	24:	40	Y	1.10	100.00	
1,2,3,4,6,7,8-HpCDF	37429000	1.04	Y	24:	41	Y	1.16	100.00	0.057
1,2,3,4,7,8,9-HpCDF	31984000	1.05	Y	26:	2	Y	0.99	100.00	0.067
Total HPCDF							1.07		
13C-1,2,3,4,6,7,8-HpCDD	25894000	1.09	Y	25:	37	Y	0.87	100.00	
1,2,3,4,6,7,8-HpCDD	26807000	1.04	Y	25:	38	Y	1.04	100.00	0.005
Total HPCDD							1.04		
13C-OCDD	36893000	0.89	Y	28:	7	Y	0.62	200.00	
OCDF	49874000	0.92	Y	28:	14	Y	1.36	200.00	0.000
OCDD	40566000	0.89	Y	28:	7	Y	1.11	200.00	0.000

TABLE 6 - Dioxin Furan One point Calibration Curve

Mass Spec : AUTOSPEC
GC Column : DB5

File name 7FEB21.REF
One Point Calibration Curve
INITIAL CALIBRATION CURVE

		Mean	S.D.	%RSD	1	2	3	4	5
13C-2,3,7,8-TCDF	Amount				100.00				
	RF				156.34				
	RRF	1.56	0.000	0.000	1.56				
2,3,7,8-TCDF	Amount				20.00				
	RF				18.81				
	RRF	0.94	0.000	0.000	0.94				
Total TCDF	Amount				20.00				
	RF				18.81				
	RRF	0.94	0.000	0.000	0.94				
13C-2,3,7,8-TCDD	Amount				100.00				
	RF				93.24				
	RRF	0.93	0.000	0.000	0.93				
2,3,7,8-TCDD	Amount				20.00				
	RF				20.58				
	RRF	1.03	0.000	0.000	1.03				
Total TCDD	Amount				20.00				
	RF				20.58				
	RRF	1.03	0.000	0.000	1.03				
13C-1,2,3,7,8-PeCDF	Amount				100.00				
	RF				117.50				
	RRF	1.18	0.000	0.000	1.18				
1,2,3,7,8-PeCDF	Amount				100.00				
	RF				122.42				
	RRF	1.22	0.000	0.000	1.22				
2,3,4,7,8-PeCDF	Amount				100.00				
	RF				124.92				
	RRF	1.25	0.000	0.000	1.25				
Total PeCDF	Amount				100.00				
	RF				123.67				
	RRF	1.24	0.000	0.000	1.24				
13C-1,2,3,7,8-PeCDD	Amount				100.00				
	RF				70.70				
	RRF	0.71	0.000	0.000	0.71				
1,2,3,7,8-PeCDD	Amount				100.00				
	RF				111.01				
	RRF	1.11	0.000	0.000	1.11				
Total PeCDD	Amount				100.00				
	RF				111.01				
	RRF	1.11	0.000	0.000	1.11				
13C-1,2,3,4,7,8-HxCDF	Amount				100.00				
	RF				100.99				
	RRF	1.01	0.000	0.000	1.01				
1,2,3,4,7,8-HxCDF	Amount				100.00				
	RF				132.85				
	RRF	1.33	0.000	0.000	1.33				
1,2,3,6,7,8-HxCDF	Amount				100.00				
	RF				166.84				
	RRF	1.67	0.000	0.000	1.67				

Table 6 (cont'd)		Mean	S.D.	%RSD	1	2	3	4	5
1,2,3,7,8,9-HxCDF	Amount				100.00				
	RF				137.42				
	RRF	1.37	0.000	0.000	1.37				
2,3,4,6,7,8-HxCDF	Amount				100.00				
	RF				113.48				
	RRF	1.13	0.000	0.000	1.13				
Total HxCDF	Amount				100.00				
	RF				137.65				
	RRF	1.38	0.000	0.000	1.38				
13C-1,2,3,6,7,8-HxCDD	Amount				100.00				
	RF				96.61				
	RRF	0.97	0.000	0.000	0.97				
1,2,3,4,7,8-HxCDD	Amount				100.00				
	RF				77.17				
	RRF	0.77	0.000	0.000	0.77				
1,2,3,6,7,8-HxCDD	Amount				100.00				
	RF				102.35				
	RRF	1.02	0.000	0.000	1.02				
1,2,3,7,8,9-HxCDD	Amount				100.00				
	RF				91.69				
	RRF	0.92	0.000	0.000	0.92				
Total HxCDD	Amount				100.00				
	RF				90.40				
	RRF	0.90	0.000	0.000	0.90				
13C-1,2,3,4,6,7,8-HpCDF	Amount				100.00				
	RF				109.83				
	RRF	1.10	0.000	0.000	1.10				
1,2,3,4,6,7,8-HpCDF	Amount				100.00				
	RF				115.78				
	RRF	1.16	0.000	0.000	1.16				
1,2,3,4,7,8,9-HpCDF	Amount				100.00				
	RF				98.88				
	RRF	0.99	0.000	0.000	0.99				
Total HpCDF	Amount				100.00				
	RF				107.33				
	RRF	1.07	0.000	0.000	1.07				
13C-1,2,3,4,6,7,8-HpCDD	Amount				100.00				
	RF				87.29				
	RRF	0.87	0.000	0.000	0.87				
1,2,3,4,6,7,8-HpCDD	Amount				100.00				
	RF				103.55				
	RRF	1.04	0.000	0.000	1.04				
Total HpCDD	Amount				100.00				
	RF				103.55				
	RRF	1.04	0.000	0.000	1.04				
13C-OCDD	Amount				200.00				
	RF				124.66				
	RRF	0.62	0.000	0.000	0.62				
OCDF	Amount				200.00				
	RF				271.84				
	RRF	1.36	0.000	0.000	1.36				
OCDD	Amount				200.00				
	RF				221.11				
	RRF	1.11	0.000	0.000	1.11				

TABLE 7 - Result Table

Weight : 1 Name	Total Response	Isotope Ratio		R. mm:	T. ss		RRF	pg	Rec/ MDL
PCB-112	98894000	1.55	Y	14:	11	Y	1.00		
13C-1,2,3,4-TCDD	28432000	0.8	Y	17:	3	Y	1.00	100.00	102
13C-2,3,7,8-TCDF	45369000	0.78	Y	16:	59	Y	1.56	102.07	0.167
2,3,7,8-TCDF	* No Peak	0	N	16:	56	Y	0.94	0.00	
Total TCDF	* No Peak *						0.94	0.00	
13C-2,3,7,8-TCDD	27095000	0.79	Y	17:	21	Y	0.93	102.21	102
2,3,7,8-TCDD	* No Peak	0	N	17:	21	Y	1.03	0.00	0.816
Total TCDD	* No Peak *						1.03	0.00	
13C-1,2,3,7,8-PeCDF	34670000	1.66	Y	19:	21	Y	1.18	103.78	104
1,2,3,7,8-PeCDF	* No Peak	0	N	19:	22	Y	1.22	0.00	0.063
2,3,4,7,8-PeCDF	* No Peak	0	N	20:	2	N	1.25	0.00	0.062
Total PECDF	* No Peak *						1.24	0.00	
13C-1,2,3,7,8-PeCDD	19257600	1.73	Y	20:	15	Y	0.71	95.81	96
1,2,3,7,8-PeCDD	* No Peak	0	N	20:	15	Y	1.11	0.00	0.370
Total PECDD	* No Peak *						1.11	0.00	
13C-1,2,3,7,8,9-HxCDD	27372000	1.3	Y	23:	9	Y	1.00	100.00	
13C-1,2,3,4,7,8-HxCDF	24758100	0.52	Y	22:	9	y	1.01	89.56	90
1,2,3,4,7,8-HxCDF	* No Peak	0	N	22:	9	Y	1.33	0.00	0.309
1,2,3,6,7,8-HxCDF	* No Peak	0	N	22:	14	N	1.67	0.00	0.246
1,2,3,7,8,9-HxCDF	* No Peak	0	N	22:	44	N	1.37	0.00	0.299
2,3,4,6,7,8-HxCDF	* No Peak	0	N	23:	23	N	1.13	0.00	0.362
Total HXCDF	8667						1.38	0.03	
13C-1,2,3,6,7,8-HxCDD	27913000	1.32	Y	22:	58	Y	0.97	105.56	106
1,2,3,4,7,8-HxCDD	* No Peak	0	N	22:	53	N	0.77	0.00	0.069
1,2,3,6,7,8-HxCDD	11614	0.71	N	22:	59	N	1.02	0.04	0.025
1,2,3,7,8,9-HxCDD	18554	0.19	N	23:	11	Y	0.92	0.07	0.027
Total HXCDD	5269						0.90	0.02	
13C-1,2,3,4,6,7,8-HpCDF	28987000	1	Y	24:	39	Y	1.10	96.36	96
1,2,3,4,6,7,8-HpCDF	9465	0.7	N	24:	38	Y	1.16	0.03	0.003
1,2,3,4,7,8,9-HpCDF	5118	1.26	Y	26:	0	Y	0.99	0.02	0.003
Total HPCDF	5118						1.07	0.02	
13C-1,2,3,4,6,7,8-HpCDD	20956000	1.09	Y	25:	37	Y	0.87	87.71	88
1,2,3,4,6,7,8-HpCDD	10466	2.47	N	25:	38	Y	1.04	0.05	0.013
Total HPCDD	No Peak *						1.04	0.00	
13C-OCDD	33638000	0.89	Y	28:	6	Y	0.62	197.17	99
OCDF	21356	0.39	N	28:	15	Y	1.36	0.09	0.000
OCDD	46021	0.53	N	28:	8	Y	1.11	0.25	0.000

TABLE 8 - Dioxin Furan Target

21-FEB-1997 01:11:23 pm Dioxin Furan Ical TARGETS

Targets : DF7FEB.TRG

21-FEB-1997 01:08:30 pm

is /A	Mass (1)	Mass (2)	ml /m2	Tol. (%)	Amt. pg	R. mm:	T. ss	Tol. Sec.	Name
K	326.000	328.000	1.55	25.0	200.00	14:	11	20	PCB-112
R	332.000	334.000	0.77	25.0	100.00	17:	3	5	13C-1,2,3,4-TCDD
I	316.000	318.000	0.77	25.0	100.00	16:	58	10	13C-2,3,7,8-TCDF
A	304.000	306.000	0.77	25.0	20.00	16:	58	5	2,3,7,8-TCDF
T	304.000	306.000	0.77	25.0					Total TCDF
B									
I	332.000	334.000	0.77	25.0	100.00	17:	21	5	13C-2,3,7,8-TCDD
A	320.000	322.000	0.77	25.0	20.00	17:	21	5	2,3,7,8-TCDD
T	320.000	322.000	0.77	25.0					Total TCDD
B									
I	352.000	354.000	1.55	25.0	100.00	19:	22	10	13C-1,2,3,7,8-PeCDF
A	340.000	342.000	1.55	25.0	100.00	19:	22	5	1,2,3,7,8-PeCDF
A	340.000	342.000	1.55	25.0	100.00	20:	2	5	2,3,4,7,8-PeCDF
T	340.000	342.000	1.55	25.0					Total PECDF
B									
I	368.000	370.000	1.55	25.0	100.00	20:	15	10	13C-1,2,3,7,8-PeCDD
A	356.000	358.000	1.55	25.0	100.00	20:	15	5	1,2,3,7,8-PeCDD
T	356.000	358.000	1.55	25.0					Total PECDD
B									
KR	402.000	404.000	1.24	30.0	100.00	23:	10	10	13C-1,2,3,7,8,9-HxCDD
I	384.000	386.000	0.51	30.0	100.00	22:	10	5	13C-1,2,3,4,7,8-HxCDF
A	374.000	376.000	1.24	30.0	100.00	22:	10	5	1,2,3,4,7,8-HxCDF
A	374.000	376.000	1.24	30.0	100.00	22:	15	5	1,2,3,6,7,8-HxCDF
A	374.000	376.000	1.24	30.0	100.00	22:	45	5	1,2,3,7,8,9-HxCDF
A	374.000	376.000	1.24	30.0	100.00	23:	24	5	2,3,4,6,7,8-HxCDF
T	374.000	376.000	1.24	25.0					Total HXCDF
B									
I	402.000	404.000	1.24	25.0	100.00	22:	58	10	13C-1,2,3,6,7,8-HxCDD
A	390.000	392.000	1.24	25.0	100.00	22:	54	5	1,2,3,4,7,8-HxCDD
A	390.000	392.000	1.24	25.0	100.00	22:	58	5	1,2,3,6,7,8-HxCDD
A	390.000	392.000	1.24	25.0	100.00	23:	10	5	1,2,3,7,8,9-HxCDD
T	390.000	392.000	1.24	25.0					Total HXCDD
B									
I	420.000	422.000	1.04	25.0	100.00	24:	40	10	13C-1,2,3,4,6,7,8-HpCDF
A	408.000	410.000	1.04	25.0	100.00	24:	40	5	1,2,3,4,6,7,8-HpCDF
A	408.000	410.000	1.04	25.0	100.00	26:	2	5	1,2,3,4,7,8,9-HpCDF
T	408.000	410.000	1.04	25.0					Total HPCDF
B									
I	436.000	438.000	1.04	25.0	100.00	25:	38	10	13C-1,2,3,4,6,7,8-HpCDD
A	424.000	426.000	1.04	30.0	100.00	25:	38	5	1,2,3,4,6,7,8-HpCDD
T	424.000	426.000	1.04	25.0					Total HPCDD
B									
I	470.000	472.000	0.89	25.0	200.00	28:	7	10	13C-OCDD
A	442.000	444.000	0.89	25.0	200.00	28:	14	5	OCDF
A	458.000	460.000	0.89	25.0	200.00	28:	7	5	OCDD

TABLE 9 - Dioxin Furan Five-point Calibration Curve

Mass Spec : AUTOSPEC
GC Column : DB5

File name CAL5JUL.RRF
Dioxin Furan Five Point Calibration Curve
INITIAL CALIBRATION CURVE

		Mean	S.D.	%RSD	1	2	3	4	5
13C-2,3,7,8-TCDF	Amount				50.00	50.00	50.00	50.00	50.00
	RF				73.75	83.75	82.01	79.44	78.67
	RRF	1.59	0.076	4.793	1.47	1.68	1.64	1.59	1.57
2,3,7,8-TCDF	Amount				2.50	5.00	10.00	20.00	40.00
	RF				2.58	4.50	9.91	20.86	40.29
	RRF	0.99	0.057	5.699	1.03	0.90	0.99	1.04	1.01
Total TCDF	Amount				2.50	5.00	10.00	20.00	40.00
	RF				2.58	4.50	9.91	20.86	40.29
	RRF	0.99	0.057	5.699	1.03	0.90	0.99	1.04	1.01
13C-2,3,7,8-TCDD	Amount				50.00	50.00	50.00	50.00	50.00
	RF				43.83	45.48	45.11	44.20	45.73
	RRF	0.90	0.016	1.830	0.88	0.91	0.90	0.88	0.91
2,3,7,8-TCDD	Amount				2.50	5.00	10.00	20.00	40.00
	RF				2.67	4.98	10.75	22.88	45.04
	RRF	1.08	0.058	5.343	1.07	1.00	1.08	1.14	1.13
Total TCDD	Amount				2.50	5.00	10.00	20.00	40.00
	RF				2.67	4.98	10.75	22.88	45.04
	RRF	1.08	0.058	5.343	1.07	1.00	1.08	1.14	1.13
13C-1,2,3,7,8-PeCDF	Amount				50.00	50.00	50.00	50.00	50.00
	RF				53.09	65.34	66.17	64.82	62.86
	RRF	1.25	0.107	8.606	1.06	1.31	1.32	1.30	1.26
1,2,3,7,8-PeCDF	Amount				12.50	25.00	50.10	100.00	200.00
	RF				13.31	25.30	51.92	109.85	222.17
	RRF	1.06	0.041	3.856	1.06	1.01	1.04	1.10	1.11
2,3,4,7,8-PeCDF	Amount				12.50	25.00	50.00	100.00	200.00
	RF				13.02	26.87	52.32	108.48	219.22
	RRF	1.07	0.024	2.238	1.04	1.07	1.05	1.08	1.10
Total PeCDF	Amount				12.50	25.00	50.00	100.00	200.00
	RF				13.16	26.08	52.12	109.16	220.69
	RRF	1.07	0.029	2.689	1.05	1.04	1.04	1.09	1.10
13C-1,2,3,7,8-PeCDD	Amount				50.00	50.00	50.00	50.00	50.00
	RF				25.88	34.97	33.84	34.18	33.73
	RRF	0.65	0.075	11.506	0.52	0.70	0.68	0.68	0.67
1,2,3,7,8-PeCDD	Amount				12.50	25.00	50.00	100.00	200.00
	RF				14.20	25.21	55.27	107.69	219.98
	RRF	1.09	0.048	4.404	1.14	1.01	1.11	1.08	1.10
Total PeCDD	Amount				12.50	25.00	50.00	100.00	200.00
	RF				14.20	25.21	55.27	107.69	219.98
	RRF	1.09	0.048	4.404	1.14	1.01	1.11	1.08	1.10
13C-1,2,3,4,7,8-HxCDF	Amount				50.00	50.00	50.00	50.00	50.00
	RF				60.89	62.30	56.78	60.98	57.21
	RRF	1.19	0.049	4.150	1.22	1.25	1.14	1.22	1.14
1,2,3,4,7,8-HxCDF	Amount				12.50	25.00	50.00	100.00	200.00
	RF				15.74	27.95	59.56	121.44	243.31
	RRF	1.20	0.052	4.329	1.26	1.12	1.19	1.21	1.22
1,2,3,6,7,8-HxCDF	Amount				12.50	25.00	50.00	100.00	200.00
	RF				18.05	35.88	75.03	158.03	301.71
	RRF	1.49	0.058	3.912	1.44	1.44	1.50	1.58	1.51

Table 9 - *cont'd*		**Mean**	**S.D.**	**%RSD**	**1**	**2**	**3**	**4**	**5**
1,2,3,7,8,9-HxCDF	Amount				12.50	25.00	50.00	100.00	200.00
	RF				13.82	26.96	56.08	112.66	229.07
	RRF	1.12	0.025	2.260	1.11	1.08	1.12	1.13	1.15
2,3,4,6,7,8-HxCDF	Amount				12.50	25.00	50.00	100.00	200.00
	RF				11.26	19.86	45.88	88.49	187.46
	RRF	0.89	0.055	6.240	0.90	0.79	0.92	0.88	0.94
Total HxCDF	Amount				12.50	25.00	50.00	100.00	200.00
	RF				14.72	27.66	59.14	120.16	240.39
	RRF	1.17	0.039	3.353	1.18	1.11	1.18	1.20	1.20
13C-1,2,3,6,7,8-HxCDD	Amount				50.00	50.00	50.00	50.00	50.00
	RF				57.78	62.14	59.27	61.74	58.17
	RRF	1.20	0.040	3.372	1.16	1.24	1.19	1.23	1.16
1,2,3,4,7,8-HxCDD	Amount				12.50	25.00	50.00	100.00	200.00
	RF				8.92	17.84	32.82	69.17	150.38
	RRF	0.71	0.035	4.954	0.71	0.71	0.66	0.69	0.75
1,2,3,6,7,8-HxCDD	Amount				12.50	25.00	50.00	100.00	200.00
	RF				11.71	21.72	46.97	93.83	181.51
	RRF	0.92	0.031	3.347	0.94	0.87	0.94	0.94	0.91
1,2,3,7,8,9-HxCDD	Amount				12.50	25.00	50.00	100.00	200.00
	RF				8.08	16.22	37.29	75.78	163.53
	RRF	0.72	0.074	10.261	0.65	0.65	0.75	0.76	0.82
Total HxCDD	Amount				12.50	25.00	50.00	100.00	200.00
	RF				9.57	18.59	39.03	79.59	165.14
	RRF	0.78	0.031	3.960	0.77	0.74	0.78	0.80	0.83
13C-1,2,3,4,6,7,8-HpCDF	Amount				50.00	50.00	50.00	50.00	50.00
	RF				60.61	59.06	56.42	64.07	65.12
	RRF	1.22	0.072	5.864	1.21	1.18	1.13	1.28	1.30
1,2,3,4,6,7,8-HpCDF	Amount				12.50	25.00	50.00	100.00	200.00
	RF				14.43	26.88	57.32	110.46	224.72
	RRF	1.12	0.032	2.869	1.15	1.08	1.15	1.10	1.12
1,2,3,4,7,8,9-HpCDF	Amount				12.50	25.00	50.00	100.00	200.00
	RF				10.97	19.68	38.67	80.39	173.08
	RRF	0.82	0.047	5.728	0.88	0.79	0.77	0.80	0.87
Total HpCDF	Amount				12.50	25.00	50.00	100.00	200.00
	RF				12.70	23.28	48.00	95.43	198.90
	RRF	0.97	0.034	3.476	1.02	0.93	0.96	0.95	0.99
13C-1,2,3,4,6,7,8-HpCDD	Amount				50.00	50.00	50.00	50.00	50.00
	RF				44.44	39.74	38.82	41.30	48.68
	RRF	0.85	0.080	9.123	0.89	0.79	0.78	0.83	0.97
1,2,3,4,6,7,8-HpCDD	Amount				12.50	25.00	50.00	100.00	200.00
	RF				11.83	26.10	49.61	105.83	199.69
	RRF	1.01	0.045	4.417	0.95	1.04	0.99	1.06	1.00
Total HpCDD	Amount				12.50	25.00	50.00	100.00	200.00
	RF				11.83	26.10	49.61	105.83	199.69
	RRF	1.01	0.045	4.417	0.95	1.04	0.99	1.06	1.00
13C-OCDD	Amount				100.00	100.00	100.00	100.00	100.00
	RF				71.49	70.81	68.01	78.68	87.49
	RRF	0.75	0.079	10.453	0.71	0.71	0.68	0.79	0.87
OCDF	Amount				25.00	50.00	100.00	200.00	400.00
	RF				28.32	56.43	120.68	245.51	498.28
	RRF	1.19	0.054	4.572	1.13	1.13	1.21	1.23	1.25
OCDD	Amount				25.00	50.00	100.00	200.00	400.00
	RF				24.22	50.43	102.39	209.61	432.81
	RRF	1.03	0.042	4.136	0.97	1.01	1.02	1.05	1.08

10. QUALITY CONTROL

10.1. HRMS Resolution

Static resolving power checks are performed daily with a PFK solution as described in Section 7.10. If the required sensitivity cannot be achieved, the inner ion source is cleaned, or the ceramic lines in the interface are replaced (SOP-CHEM-PROC-11). If the required sensitivity still cannot be achieved, a service call is placed to the manufacturer.

10.2. Calibration Verification

System performance and calibration are verified daily as described in 7.11. The calculated concentration for each native congener must be within 20% of its actual known value. The calculated recovery of each surrogate compound must be within the range of 75 to 120%. If any compound fails its respective limit, a fresh calibration standard is prepared or the problem causing the failure is corrected.

10.3. Retention Times Windows and GC Resolution

For each new GC column, the optimum setting for correct retention time windows is verified by analyzing a Window Defining Mixture containing the earliest and latest eluting congeners (**Table 3**) within each homologous group of congeners. This verification is performed at regular intervals, and after any condition changes or upset that requires that the GC column be disconnected. Reset the retention time windows, when it is required.

The valley between peaks representing 2,3,7,8-TCDD and its closest neighboring isomer should be equal to or less than 25% of the 2,3,7,8,-TCDD peak height. The corresponding peak valley criterion for 2,3,7,8-TCDF is 30% maximum [2.10]. If the criteria have not been met, the results must be flagged.

The patterns of PCDDs/PCDFs/NOPCBs congener data reported in samples must conform to expected patterns. If not, then the qualitative identifications by chromatography is suspect and the raw data is re-examined and reprocessed through the computer programs. If unusual patterns are persistent, re-analysis of the sample aliquot (or a different aliquot of the same sample) is performed.

10.4. Compound Verification Criteria for Sample Analysis

Peak GC retention time must be within 10 sec of the correct retention time, determined by the Instrument Daily Calibration Verification (Section 7.11).

Peak responses for each of the two selected molecular cluster ions must be at least three times the noise level (S/N $\geq$ 3).

The chlorine isotope ratio for the two molecular cluster ions (for the majority of the compounds) must be within $\pm$ 25 % of the correct isotope ratio when a 30 m long DB-5 column is being used.

Note: The "Dioxin" software uses these criteria to perform automated peak verification, prior to calculating analyte concentration.

10.5. Ongoing Precision and Recovery

An aliquot of the QA Reference Material (Herring gull eggs - Section 4.6) is analyzed along with each batch of samples. The concentration of each congener is determined and the results are compared to the previously established acceptance limits (i.e., $\pm$ 2 SD of the long-term mean plotted in a Shewart chart - ref. SOP-CHEM-DOC-02).

Because each individual sample as well as the QA Reference Material is spiked with labeled surrogates, it is possible to determine the recovery of each compound of interest. It is assumed that the recovery of labeled compounds is the same as native ones naturally incurred in the sample. If the recovery is less than 40% for the majority of the surrogates then the analysis is repeated, subject to availability of the sample material. If several samples in a batch have consistently low recoveries then an investigation of the method is done.

The recovery for each of the labeled congeners in every analysis (tissue sample, QA Reference Material, Blank) should be within the range of 80 to 120% of the spiked value (i.e., accuracy of $\pm$ 20%) [2.10]. Acceptable recoveries in tissues have been defined as 40 to 120% by the Dioxin Quality Assurance Advisory Committee (DQAAC): "Although individual surrogate recoveries as low as 30 or 40% will be considered acceptable, consistently low or highly variable recoveries may indicate that one or more of the sample processing procedures, or the GC/MS instrumentation, is not effectively controlled" [2.10].

10.6. Method Blank

A chicken egg lipid spiked with labeled surrogates is analyzed with each sample batch, to demonstrate freedom from cross-contamination and contaminants, that would interfere with PCDD/PCDF/NOPCB analysis.

10.7. Standard Operating Procedures

Other related SOPs relevant to this analytical method:

- *SOP-CHEM-DOC-01*: for the use of Log-Books
- *SOP-CHEM-DOC-02*: for the creation of control charts for Quality Control Samples
- *SOP-CHEM-PROC-01*: for pipets calibration
- *SOP-CHEM-PROC-03*: for balances calibration
- *SOP-CHEM-PROC-05* for the preparation and storage of standard solutions
- *SOP-CHEM-PROC-06*: for the monitoring of refrigerator's temperatures
- *SOP-CHEM-PROC-08*: for the archival of data files and analytical test reports
- *SOP-CHEM-PROC-09*: for glassware cleaning
- *SOP-CHEM-PROC-11*: for the tuning and calibration of the HRGC/HRMS
- *SOP-CHEM-PROC-13*: for verification of standard with a second source standard
- *SOP-CHEM-MAIN-05*: for the maintenance of the HRMS

10.8. Data Validation

Data validation is insured by an internal quality assurance audit done by an independent reviewer (Head of the Laboratory Services Section), before the release of the analytical test report. Results of this verification are recorded on the "Data Validation Form for PCDD/PCDF/noPCB Reports".

If large discrepancies in the analytical data between the specimens from close geographical areas are noted, then the raw data are examined - re-analysis of the sample aliquot may be indicated.

10.9. Method Validation

Method has been validated by multiple analysis of chicken lipid spiked at two concentrations of PCDDs/PCDFs. Repeatability tests concerning the recovery of PCDD and PCDF spikes from egg substrates are summarized in Norstrom and Simon (1991) [2.3]. Internal standard recovery is usually better than 80%.

11. Critical Control Points

Sample extracts must not be allowed to evaporate to dryness at any of the clean-up steps, since OCDD may absorb to the glass and cannot be recovered, also trichloro- and tetrachloro-NOPCB are volatile, and evaporating the sample extracts to dryness will result in loss of these compounds.

Trace contaminant levels (less than 1 ng/kg) are determined by this method and the elimination of interferences is essential. They could occur through sample handling, reagents, solvents, instruments or labware.